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## Alterations in Peritoneum Exposed to High-Energy Laser Radiation

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The effect of carbon dioxide (CO<sub>2</sub>) and Nd-YAG high-energy lasers on the formation of peritoneal adhesions was studied using light, scanning, and transmission electron microscopy in albino rats. Both types of lasers initiate the development of peritoneal adhesions similar in structure. The intensity of adhesion formation is markedly lower in the case of Nd-YAG laser as compared to the action of CO<sub>2</sub> laser of the same power.

**Key Words:** peritoneum; high-energy laser; adhesions

Laser scalpels based on high-energy radiation sources rank among the finest surgical tools. They offer advantages over the usual scalpels due to their hemostatic, aseptic, and other properties, and hence have made it possible to improve or even change the techniques of surgical treatment [2,5,7,8,10,11].

Morphological studies of reparative processes in parenchymatous organs after laser treatment have shown that healing of the incisions made by these instruments occurs more rapidly and with fewer complications, such as suppuration, tearing of sutures, etc. [1,3]. However, such complications as the formation of adhesions and the development of so-called adhesion disease [4,6,10,12] have not discouraged the use of laser scalpels in abdominal surgery. Initiation of adhesion development may above all occur

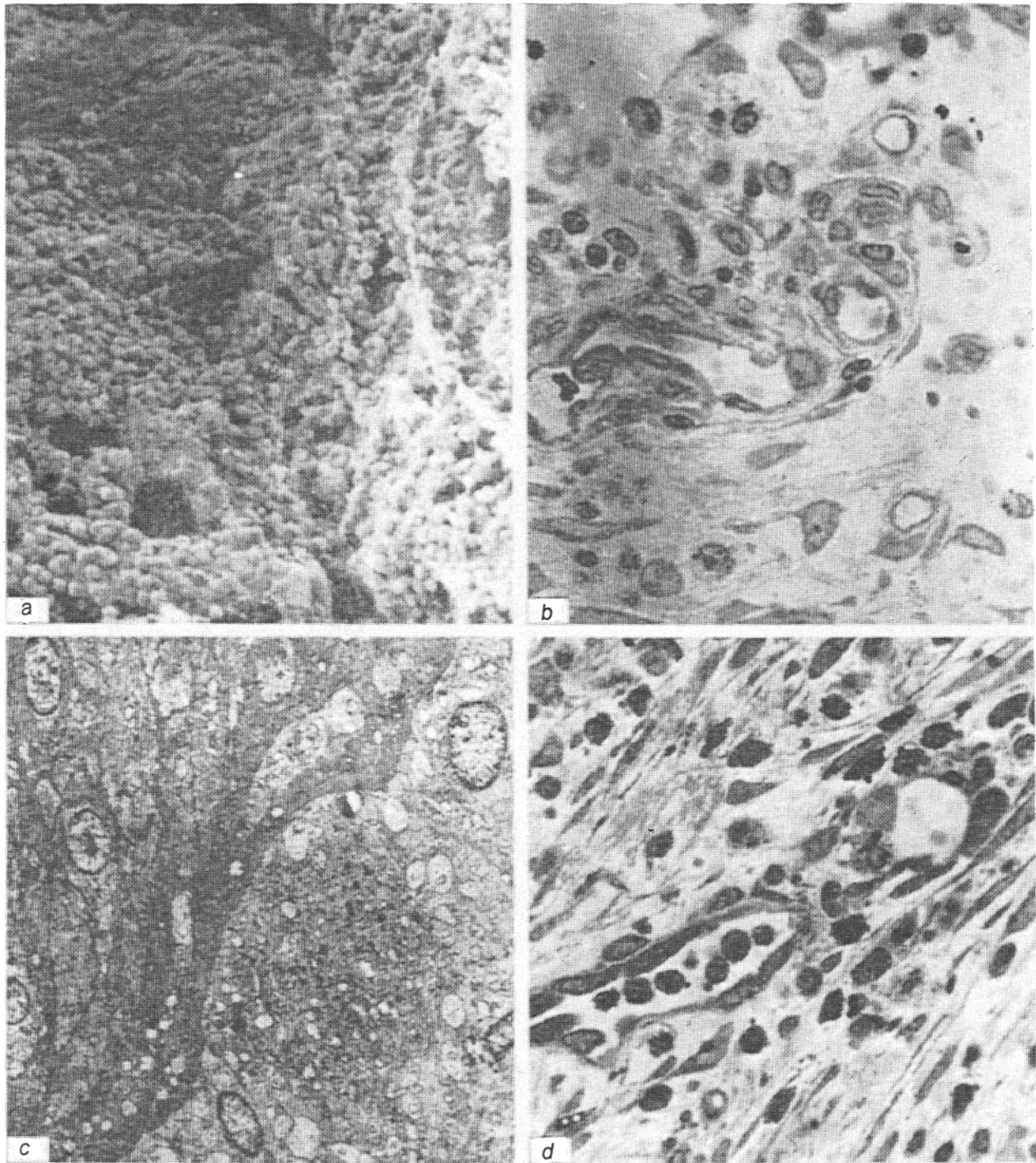
due to alteration of the serous membranes, particularly destruction of mesothelium integrity. However, the changes occurring in serous membranes and especially their mesothelial lining exposed to high-energy lasers have not been studied.

The goal of the present investigation was to study the alterations of serous membranes from different intestinal regions following the use of CO<sub>2</sub> and Nd-YAG lasers.

### MATERIALS AND METHODS

Albino rats under Nembutal anesthesia were subjected to median laparotomy and superficial laser wounds were inflicted on intestinal loops and parietal peritoneum. The animals were sacrificed 3, 7, and 14 day after the operation. Pieces of peritoneum from the adhesion region were examined morphologically with light, transmission (TEM), and scanning (SEM) electron microscopy.

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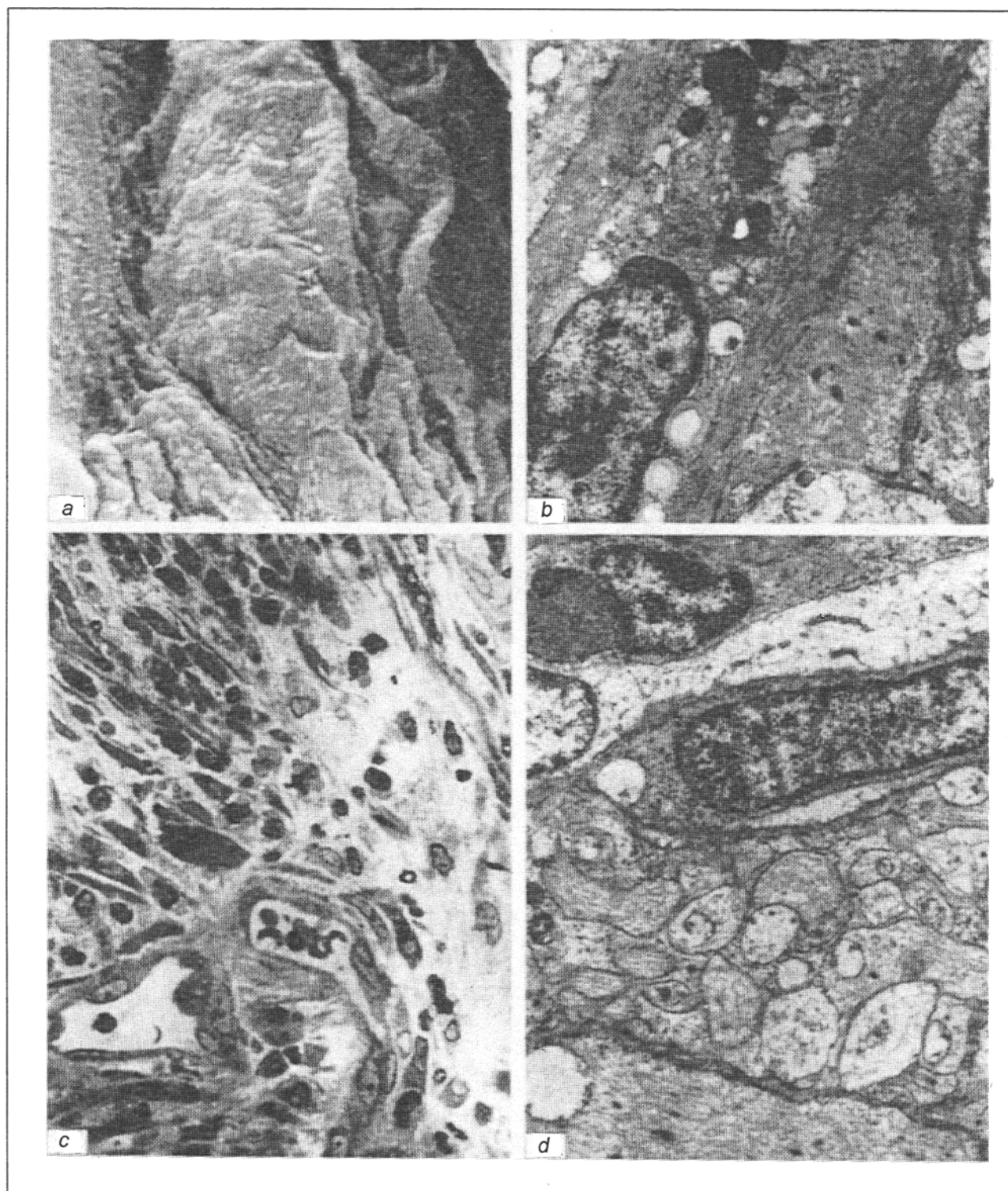


**Fig. 1.** Structure of peritoneal adhesions after high-energy laser exposure. *a*) region of CO<sub>2</sub> laser action, clusters of peritoneal fluid cells on surface of peritoneal defect. SEM,  $\times 1000$ ; *b*) capillaries, little-differentiated cells, and young fibroblasts inside an adhesion 3 days after CO<sub>2</sub> laser action. Semithin sections stained with methylene blue and fuchsin,  $\times 40$ ; *c*) macrophages and lymphoid cells inside an adhesion close to the middle coat of the small intestine, 3 days after Nd-YAG laser action. TEM,  $\times 1500$ ; *d*) increased content of fibroblasts of collagen fibers inside an adhesion, 7 days after CO<sub>2</sub> laser action. Semithin section stained with methylene blue and fuchsin,  $\times 400$ .

## RESULTS

The tests showed that 3 days after CO<sub>2</sub> laser exposure adhesions predominantly develop between loops of bowel

in the peritoneal cavity. The adhesion surface is lined with mesothelial cells of oblong shape, the apical portion of whose membrane protrudes into the lumen of the peritoneal cavity and forms a large number of microvilli. Defects



**Fig. 2.** Structure of peritoneal adhesions after high-energy laser exposure. a) mesothelial cells covering adhesion surface, 7 days after CO<sub>2</sub> laser action. SEM,  $\times 200$ ; b) smooth muscle cells inside an adhesion, widening of perinuclear spaces, decrease of myofilament content in cytoplasm, 7 days after CO<sub>2</sub> laser action. TEM,  $\times 5000$ ; c) bundle of smooth muscle cells inside an adhesion, 14 days after Nd-YAG laser action. Semithin section stained with methylene blue and fuchsin,  $\times 400$ ; d) bundles of unmyelinated nerve fibers inside an adhesion, 14 days after CO<sub>2</sub> laser action. TEM,  $\times 400$ .

of the mesothelial lining are occasionally revealed in the zone of laser impact; the surface of such defects is covered with cells of peritoneal exudate, mostly macrophages and lymphoid cells (Fig. 1, a).

Loose connective tissue forms the matrix of adhesions, consisting of fine collagen fibers, thin-walled capillaries, and a small number of cells of the fibroblast type (Fig. 1, b). Macrophages and lymphoid cells are also present.

Three days after Nd-YAG-laser manipulation the number of adhesions in the peritoneal cavity is markedly lower than after CO<sub>2</sub> laser exposure. The adhesion matrix is again composed of loose connective tissue comprising thin bundles of collagen fibers, a small number of thin-walled capillaries, and a relatively low number of fibroblasts of oblong shape with a well-developed granular endoplasmic reticulum and Golgi apparatus. Macrophages and lymphoid cells are found inside the adhesions (Fig. 1, c).

Seven days after CO<sub>2</sub> laser exposure the structure of the adhesions is altered as follows: they consolidate, the number of collagen fibrils is markedly increased, and a large number of fibroblasts of oblong or sprig-like shape with large hyperchromic nuclei appear (Fig. 1, d). Macrophages and occasional lymphoid cells are found. The matrix of adhesions in some cases consists of dissociated spindle-shaped smooth muscle cells with a few myofilaments and deep invaginations of the nuclear membrane. Seven days after Nd-YAG laser exposure the total number of adhesions and the extent of their development are significantly lower than in the case of CO<sub>2</sub> laser action. In some animals the formation of adhesions was not noted at all. There were no cases of adhesion of the membranous part of the greater omentum to the laser-exposed zone.

According to the SEM study, the zone of peritoneal injury is covered with mesothelial cells with a surface and ultrastructural organization similar to that of the normal mesothelium which lines the undamaged part of the intestinal peritoneum (Fig. 2, a).

When adhesions formed their surface was covered with mesothelial cells the apical surface of which bore a large number of microvilli. As a rule the cellular boundaries were not revealed in the SEM study.

Examination of the semithin sections showed that the adhesion matrix consists of newly formed connective tissue with thin bundles of collagen fibrils, thin-walled widened capillaries, fibroblasts, and smooth muscle oblong cells. An insignificant number of macrophages and lymphoid cells are also found. Detailed study of oriented preparations reveals that the middle coat of the bowel is the source of the smooth muscle cells found inside the adhesions. In this case dissociation of leiomyocytes and their penetration into the connective tissue matrix of the adhesions were noted.

The ultrastructural organization of the smooth muscle cells inside the adhesions differs somewhat from that in the middle coat of the bowel. The cell cytoplasm contains fewer myofilaments; there are large vacuoles, widened cisternae of the Golgi apparatus, and a widened perinuclear space (Fig. 2, b). The cells lose their spindle-like shape, and the cytoplasmic membrane has numerous protrusions and invaginations. The nuclear membrane is invaginated.

Fourteen days after CO<sub>2</sub> laser treatment the number of peritoneal adhesions is lower than at previous times. The surface of the adhesions is lined with mesothelial cells with a flattened apical surface covered with an abundance of microvilli.

The structure of adhesions differs markedly from that at previous times as well. Their matrix is of mature connective tissue consisting of bundles of collagen fibers, fibroblasts, and capillaries with dilated lumens. There is an increased content of smooth muscle cells forming clusters and oriented perpendicularly to the lumen of the bowel (Fig. 2, c). Nerve fibers, predominantly, unmyelinated, occurring within the adhesions are typical of this period of observation. Vacuolization of the axoplasm is noted in certain nerve fibers. The ultrastructural study attested that the cytoplasm of smooth muscle cells contains myofilaments, and small mitochondria with a few cristae and electron-dense matrix. Vacuolization of the cytoplasm and widening of the Golgi apparatus cisternae are found in individual cells. Fourteen days after Nd-YAG laser action occasional adhesions are found in the peritoneal cavity between loops of the small intestine.

The surface of adhesions is lined with mesothelial cells mounted on mature connective tissue inside which capillaries, fibroblasts, and smooth muscle cells are located. As in the case of CO<sub>2</sub> laser, unmyelinated nerve fibers appear inside the adhesions (Fig. 2, d).

Thus, the study showed that the action of both types of high-energy lasers induces the formation of adhesions of similar structure, but the intensity of adhesion formation is markedly lower in the case of Nd-YAG laser as compared to carbon dioxide laser of the same power.

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